THE BEHAVIOR OF THE ACCESSORY CHROMOSOMES AND OF THE CHROMATOID BODY IN THE SPERMATOGENESIS OF THE RABBIT.[1]

L. J. BACHHUBER,

UNIVERSITY OF WISCONSIN, DEPARTMENT OF EXPERIMENTAL BREEDING.

The following studies resulted from a series of experiments to determine the effect of lead poisoning upon the germ-cells of the male rabbit as indicated by his offspring. My intention originally was to attempt to determine the manner in which the lead-poisoning affected the normal mitosis. The problem of the normal mitosis in itself proved to be so large that the study of the effect of lead-poisoning had to be postponed to a future time.

The following work was done under the direction of Dr. M. F. Guyer, to whom the writer is very much indebted for many valuable criticisms and kindly help. The writer is also indebted to the kindness of Professor L. J. Cole for aid given in getting the necessary material for this study.

All of the rabbit testes used were from animals raised at the barns of the Department of Experimental Breeding of the University of Wisconsin. These males were chosen from the normal stock resulting from the double matings described by Cole and Bachhuber (1914).

A fairly successful fixing reagent was found in Flemming's strong. This method brought out the chromosomal and cytoplasmic structures better than Gilson's, Zenker's, Hermann's and possibly Bouin's fixative. Bouin's gave good results in the study of the accessory chromosomes and the chromatoid body. A new method reported to be in use in McClung's laboratory was also tried with considerable success. This fixative employs urea as a means of more rapid penetration. To one hundred cubic centimeters of Bouin's, made up of seventy-five parts of

[1] Papers from the Department of Experimental Breeding, Wisconsin Agricultural Experiment Station, No. 6. Published with the approval of the Director of the Station.

aqueous picric acid with fifteen parts formalin and ten parts glacial acetic acid, were added one and one-half grams chromic acid and three grams of urea. The solution was then heated to thirty-seven degrees and the tissue added. This fixative made the chromosomes stand out somewhat better and clearer than any of the other fluids.

The sections were cut from four to fifteen micra in thickness and stained in (1) Delafield's hæmatoxylin and eosin, (2) Haidenhain's iron-hæmatoxylin and acid fuchsin or orange G, (3) safranin with gentian violet or lichtgrün. Method (2) proved to be the most successful and gave some very excellent results. This method was most valuable in bringing out the detailed structures, especially the chromatoid body. Cytoplasmic structures were as a rule satisfactorily stained in Flemming's triple stain.

Smear preparations were also made. These were fixed in Flemming's strong or in Bouin's fluid. Haidenhain's iron-hæmatoxylin gave the most satisfactory result when counterstained with lichtgrün.

Mammalian spermatogenesis seems to offer greater difficulties for study than any other form. This is due to the impossibility of securing by means of existing reagents and methods proper fixation. In nearly all preparations it has been found that the chromatic structures have a tendency to mass so that individual details are lost. The chromosomes in the rabbit have this to a very marked degree. The various stages in spermatogenesis were numerous enough, but a great many stages had to be examined in order to find those in which chromosome counts were possible.

N. M. Stevens (1911) in the spermatogenesis of the guinea-pig found that material "very unfavorable and the results are not so satisfactory as are desired." Montgomery (1912) in his work with the human spermatogenesis has to say practically the same thing, "the fixation was not as excellent as might be desired." Wodsedalek (1913), however, seems to have encountered less of the massing of chromosomes in the spermatogenesis of the pig than is present in other mammalian forms.

In both the rat and the guinea-pig, and later in the bull, the

writer has found the same condition, although to a lesser degree than in the rabbit, namely, the strong tendency of the parts within the nucleus to mass, hiding many of the individual structures, and leaving much to be desired in the form of improved methods. In the rabbit, the chromosomes always agglutinated, permitting but few chromosomal counts. Because of this, the only stages in which chromosomal counts were possible with any degree of accuracy were the primary spermatocytes. In later stages it was decidedly more difficult to find stages in which counts were possible.

In the rabbit, the structures to be followed more readily are, first, the two accesory chromosomes and second, the chromatoid body. After the spermatogonial stages, the accessory chromosomes could nearly always be identified and rather easily traced. The chromatoid body, similar to that described by Wilson (1913), whose origin could not be determined, can be easily followed, beginning with the primary spermatocytes, and can even be identified when it is cast off from the transforming spermatosomes.

The other structures displayed in the spermatogenesis of the rabbit, and the various processes connected with them, do not differ materially from the corresponding stages described for other forms. In the following pages, the process of spermatogenesis, in so far as it could be determined, will be taken up in the order of the successive stages of spermatogonia, primary and secondary spermatocytes, spermatosomes, and the fully developed spermatozoa.

1. Zones of Proliferation.

Throughout all the tubules of the testes, there appear small areas in which active proliferation reveals almost every stage of spermatogenesis. These areas are not, however, confined to any particular section or part of the tubule. All indications of the active zones go to show that a certain area may be active for a period, then halt in its activity while a nearby area becomes active in spermatogenesis. There are thus, scattered throughout each of the tubules, areas of active proliferation and areas of rest. In each of these areas of activity usually there

may be seen the ordinary arrangement of the spermatogonial cells on the outer margin with the primary spermatocytes adjacent to them. Further towards the center lie the secondary spermatocytes while the spermatids, the transforming spermatosomes, and the fully developed spermatozoa lie in the central cavity.

2. Spermatogonial Stages.

It has been difficult to obtain satisfactory preparations of the spermatogonial cells showing all of the essential structures, chromatic and achromatic. Nutritive cells were also comparatively scarce although a few may have been identified (Fig. 1). They are very similar to the resting stage of the spermatogonia with which they may easily be confused and their identity is never certain. The nuclei of these nutritive cells usually appear oval or irregular in shape and contain masses of chromatin scattered throughout This close resemblance to the nucleus of the resting spermatogonia makes it not at all improbable that the nutritive cells, if they are such, may be derived from the spermatogonia. Montgomery (1912) has found this to be true in man. He was able to trace directly the formation of the Sertoli cells from the spermatogonia by the presence of a rod-like body in the cytoplasm, which was, according to him, an invariable indication of the Sertoli cells. Hegner (1914) takes the view that the Sertoli cells arise from the primordial germ-cells. This may also be true in the rabbit although there is little direct evidence to support either view. The similarity of the Sertoli cells and the spermatogonia and the relative number of these cells affords the only evidence.

During the earliest prophases of the spermatogonial stage (Figs. 2, 3) the nucleus is somewhat elongated and irregular as a rule, and contains two or more large, spherical karyosomes. These may be the two accessory elements which can be traced very accurately after the formation of the primary spermatocytes. Small linin threads, somewhat granular in appearance, radiate out towards the periphery of the nuclear wall (Figs. 4, 5). Slender fibrillæ extend throughout the cytoplasm (Fig. 2). Between these are small areas of a granular appearing substance. The centrosome and the other cytoplasmic structures cannot be

identified although some of the more granular parts of the cytoplasm may include the centrosome and the chromatoid body. This is however doubtful because the staining reactions given by these structures in the primary and secondary spermatocytes ought also be given in the spermatogonia.

Only one spermatogonial stage has been found in which the chromatoid body appeared to be present (Fig. 6). While it has the characteristic appearance of this body, it is a rather doubtful case because it could not be identified in any other of the spermatogonial stages.

As development continues, dense masses of chromatin appear concentrating along the radial linin threads. These masses vary in number, but never exceed the diploid number of chromosomes, making it highly possible that these masses later transform into the univalent chromosomes. The entire nucleus stains heavily with the basic dyes, indicating a large increase of the chromatin content of the cell. In this manner, from twelve to twenty-two masses of chromatin are formed, all united by heavy linin threads. Gradually the linin threads disappear, and the chromatin masses assume a more regular appearance (Fig. 7). The nuclear wall disintegrates as the chromosomes arrange themselves in the metaphase stage (Fig. 8). The spindle fibers are very indistinct although in favorably stained sections they may be made to stand out more strongly. Because of this excess of stain, the cells which were stained heavy enough to make the spindle fibers appear more plainly were useless for the study of any of the other structures.

The chromosomes at best tend to mass together, and if not strongly destained, form a huge black mass in which nothing can be distinguished. In some of the metaphase stages it is possible to count twenty-two chromosomes (Figs. 8, 9, 10). The X and the Y elements which are shown to exist from their subsequent behavior, could not be distinguished from each other or from the remainder of the chromosomes in the spermatogonial division stage. It appears probable that the two large karyosomes present in the early spermatogonial stages may represent the accessory elements because they retain their individuality and later appear to transform directly into two

chromosomes of the spermatogonial division. At times they may be traced because of a slightly more rounded form than the other chromosomes. The chromosomes in general are elongated in shape although the tendency is towards a spherical form.

During the anaphase (Fig. 11), the divided chromosomes move toward opposite poles. As soon as the chromosomes reach the pole, they go into a resting stage, the new nuclear wall appearing immediately. The new cell walls may not appear until late in synezesis. The outlines of the new nuclei are first somewhat elongated, conforming to the rough outline of the massed chromosomes. Gradually these assume a more spherical shape, the spindle-fibers disappear, and the primary spermatocytes are ready for the growth period.

3. Early Maturation Stages.

While the number of chromosomes is not large in the rabbit, the difficulties encountered in counting chromosomes were great indeed. In all of the material, the tendency of the chromosomes to mass was, as already mentioned, very much in evidence, and the most careful tecnhique in fixing and staining did little to make the results more satisfactory. There were enough cells in which chromosome counts were possible to place the probable number as twenty-two in the spermatogonia and twelve in the primary spermatocyte.

It was found that twenty-two chromosomes went to each pole in the spermatogonial division. After synapsis has taken place, the number is twelve, showing that two of the elements remain single. These two elements are the accessory chromosomes. Thus the twenty ordinary chromosomes of the spermatogonia reduce to ten bivalents in the primary spermatocytes, while the two accessories do not undergo synapsis. As will be seen, the later behavior of these univalent chromosomes is entirely distinct from that of the others.

The early prophases of the primary spermatocytes show all but two of the chromosomes which passed to the poles in the spermatogonial division to grow irregular in shape, and finally weave out into fine strands which immediately spread throughout the nucleus (Fig. 12). All of the irregular masses spin out into

fine threads constituting the leptotene threads, leaving two, still rounded chromosomes, which can from this time on be identified as the accessories. The leptotene threads, in both sections and smears, are seen to persist as independent units. Their exact number could not be determined because of their extreme length.

During this stage, the accessory elements remain as small spherical masses which can easily be identified if the mass of leptotene threads is sufficiently destained. During the process of synapsis also, the X and the Y remain unchanged, always close together, and sometimes apparently connected by thin, dark-staining threads of chromatin material (Fig. 15). No instances have been found which at this stage show the accessory elements in different portions of the cell.

In these prophases, the presence of the chromatoid body becomes definitely established. It stains similarly to the chromatic material in the iron-hæmatoxylin, the Delafield's hæmatoxylin, and in the Flemming's triple stain. Just where this body arises is still a question. As before stated, it has been found in only one spermatogonial cell and that was a rather doubtful case. In the primary spermatocytes it is absolutely constant (Figs. 16, 20, 22). In a few rare cases, two and sometimes three have been found, but these extra bodies were always very small and disappeared in later stages. The large body can be traced through the remainder of the process of spermatogenesis. Further mention of its behavior will be made later.

In the synezesis stage (Figs. 13–16), the threads drift and mass at one pole of the cell into a grouping which has apparently no established order and from which nothing definite could be determined. Occasionally the threads seem to have a parallel arrangement suggesting parasynapsis, but when they come out of the synezesis stage, considerable evidence points to a chiasma-type synapsis (Figs. 18, 19). The threads are wound around each other, making possible a division later in which each of the haploid chromosomes would be made up of alternating segments of chromatin material, from the respective leptotene threads of the conjugating pair. That the threads still retain a constancy in number seems highly probable for in favorably stained sections where their ends can be seen, the number never exceeds

double the number, minus four, of the chromosomes of the primary spermatocyte.

Previous to the pachytene stage the cells have increased but very little in size. Now they begin to grow rapidly, complete fusion occurring between the conjugating threads, and all gradually drift back to the center of the cell, there to spread slowly throughout the nucleus. The threads enlarge, become lighter in staining capacity, due perhaps to the greater volume occupied by the pachytene thread, and finally form the large spireme (Figs. 17, 20, 21).

This stage of the primary spermatocytes, with the possible exception of the synezesis stage, persists longer than any other stage of the spermatogenesis. This conclusion is reached because these stages of the primary spermatocytes can be found in practically any portion of the tubules. Numerous as they are, it is extremely difficult to gain any knowledge of the processes in progress in them.

The accessory elements still retain their spherical form, usually lying closely side by side, but at this time occasionally in different portions of the cell (Figs. 17, 21). The chromatoid body enlarges and seems to become more prominent. At the same time it seems to acquire an activity in the cell although its function could not be determined.

4. Reduction Division.

The spireme of this stage in general appeared to be continuous, although some of the smears made under considerable pressure gave indications of segmentation (Fig. 23). The large spireme now condenses and soon forms the individual chromosomes. Up to this period of condensation the chromomeres stand out very distinctly (Figs. 18, 19). These condense to form the large mass of bivalent chromosomes which, because of their agglutination, usually stain so dense that the individual structure is entirely lost.

The X and Y elements, however, can nearly always be identified in these stages. As soon as the bivalent chromosomes are formed, they migrate into the equatorial plate. The accessory elements are nearly always a little to one side of this plate

(Fig. 23), usually close together. It may be that this position is constant, but the massing of the chromosomes hides the accessories (Fig. 24). In the division, the X and Y chromosomes move towards the poles long before the division of the ordinary bivalents (Figs. 25–29). The X is thus drawn to one pole, the Y toward the other. In this manner, each of the secondary spermatocytes will contain only eleven chromosomes, ten ordinary and one accessory (Fig. 30). The ordinary chromosomes, in their bivalent condition, are approximately twice the size of the spermatogonial chromosomes.

The split in the bivalent chromosomes is rarely in evidence except immediately preceding the division, although in a few isolated cases a V-shape becomes noticeable.

When the chromosomes reach the pole the chromatoid body seems to acquire more activity. During the formation of the equatorial plate, this body migrates close to the densely crowded chromosomes (Figs. 24–27). As soon as these start moving toward the poles, the X and Y preceding, the chromatoid body moves in between the two sets of chromosomes, lying approximately midway between the two centrosomes (Fig. 31). Just before the new cell wall is formed, this body moves into the cytoplasm of one of the newly formed spermatocytes. The possibility presents itself that the chromatoid body always follows either the X or the Y elements. The two accessories are, however, so closely related in size that it has been impossible to determine whether there is any such specific association.

Occasionally, in the division of the primary spermatocytes, there is found evidence that the accessory elements undergo a precocious division. The accessories lie in the plane of division and under certain conditions, they divide, forming two X and two Y elements. The division then proceeds in the regular manner with this exception: two X and two Y chromosomes go toward the poles, again preceding the division of the ten ordinary chromosomes (Figs. 32, 33).

5. Secondary Spermatocytes.

The division of the secondary spermatocytes proceeds without any appreciable rest stage. In the telophases of the preceding

division the nucleus is somewhat crescent shaped, but soon assumes a spherical form. This stage develops no spireme or leptotene threads. The chromosomes, eleven in number, immediately migrate into the equatorial plate to form a very dense mass which at times appears almost homogeneous, so closely are the chromosomes drawn together (Fig. 34). In this division the chromosomes, in migrating toward the poles, often appear as solid black ring-like masses with apparently no segments representing the individual elements. Dense fibers seem to be interwoven in the mass of chromosomes (Figs. 35, 36). It is seldom that the chromosomes can be distinguished in these stages. The X and the Y elements also as a rule lose their identity, but occasionally they can be identified by their presence in the center of the ring, connected by chromatin strands to the ordinary chromosomes (Figs. 37, 38).

The chromatoid body appears to undergo no division in these stages, following either one or the other of the chromatin masses. We thus find that approximately one-fourth of the total spermatids contain the chromatoid body.

6. Transformation of the Spermatids.

As soon as the division is complete, a new nuclear wall is immediately formed. The chromosomes as such disintegrate but the chromatic material soon condenses at the periphery of the nucleus (Figs. 39, 40). In this manner nearly all of the chromatic material is condensed, leaving one part which in every case is found somewhere near the center of the cell (Figs. 41, 43) and which may represent the accessory element. Sometimes there are one or more smaller bodies which may be remnants of the disintegrating ordinary chromosomes because they do not persist for any length of time. The large central body can be traced through the stages of further condensation and almost to the completely developed sperm.

After all of the chromatic material has condensed, the centrosome makes its appearance and moves close to the nucleus (Fig. 41). It here gradually becomes immeshed in strands of chromatic material within a slight infolding of the periphery of the nucleus. By a continuation of this process it soon becomes

entirely surrounded by nuclear material (Figs. 41, 42, 43). The entire nucleus then migrates to one side of the cell and gradually leaves behind all, or very nearly all, of the cytoplasm, together with the chromatoid body (Figs. 43, 44, 45). At the same time the entire nucleus begins to condense and finally forms a flat plate, which has at times, however, the appearance of a convex plate (Fig. 46). In the meantime the sperm tail has been formed from the cytoplasm with the centrosome in the neck. The nucleus, now the sperm head, gradually enlarges with a resultant diminution of staining capacity. In properly stained sections and smears there can occasionally be distinguished a number of darker staining bodies one of which is usually larger than the others. The number of these approaches very nearly the number of chromosomes which went into the sperm head. It is thus possible that the chromosomes retain their individuality even in the fully developed spermatozoa (Figs. 47, 48). As the sperm head enlarges, these dark-staining bodies gradually diffuse through the entire head (Figs. 49, 50). Further enlargement brings about the complete development of the spermatozoön. The head manifests an even staining capacity, the neck shows the presence of the centrosome, while the tail takes such a light stain that it is barely visible (Fig. 51).

7. Conclusions.

1. The number of chromosomes in the spermatogonium is probably twenty-two.

2. The number in the primary spermatocytes is placed at twelve.

3. The number in the secondary spermatocytes is placed at eleven.

4. Two accessory elements, an X and a Y, are present. One-half of the spermatozoa contain the X, and the other half, the Y element.

5. A chromatoid body is present. Its function was undetermined. It underwent no division, and was finally cast off with the excess cytoplasm in the metamorphosing spermatid.

BIBLIOGRAPHY.

Cole, L. J., and Bachhuber, L. J.

'14 The effect of lead on the germ cells of the male rabbit and fowl as indicated by their progeny. Proc. Soc. Exp. Biol. and Med., Vol. 12, No. 1, pp. 24–29.

Guyer, M. F.

'10 Accessory chromosomes in man. BIOL. BULL., Vol. 19, No. 4, pp. 219–234, pl. 1.

Hegner, R. W.

'14 Studies on germ cells. Jour. Morph., Vol. 25, No. 3, pp. 375–499, pls. 1–10.

Montgomery, T. H.

'12 Human spermatogenesis. Jour. Acad. Nat. Sci. Philadelphia, Vol. 15, 2d ser., pp. 1–22, pls. 1–4.

Stevens, N. M.

'11 Heterochromosomes in the guinea pig. BIOL. BULL., Vol. 21, No. 3, pp. 155–167.

Wilson, E. B.

'13 A chromatoid body simulating an accessory chromosome in Pentatoma. BIOL. BULL., Vol. 24, No. 6, pp. 392–410, pls. 1–3.

Wodsedalek, J. E.

'13 Spermatogenesis of the pig with special reference to the accessory chromosomes. BIOL. BULL., Vol. 25, No. 1, pp. 8–32, pls. 1–6.

Explanation of Plates.

All drawings made with camera lucida. Spencer 2 mm. apochromat oil immersion objective and × 16 compensating eye-piece used. All drawings ar- shown at a magnification of approximately 1,800 diameters. Drawings are accurate with reference to the nuclear material and the chromatoid body and their position in the cell; the cytoplasm, however, is represented conventionally. *S*, Sertoli cell; *C*, chromatoid body.

Plate I.

Fig. 1. Sertoli cell, showing the relation of the metamorphosing spermatosomes to the nurse cells.

Figs. 2, 3. Early stages of the spermatogonia with the two large karyosomes. Fig. 2 also shows the slender fibrillæ in the cytoplasm.

Figs. 4, 5. Later stages of the spermatogonial nuclei showing the linin threads radiating out towards the periphery of the nuclear wall.

Fig. 6. Shows the only spermatogonial cell in which a body was found which resembled the chromatoid body.

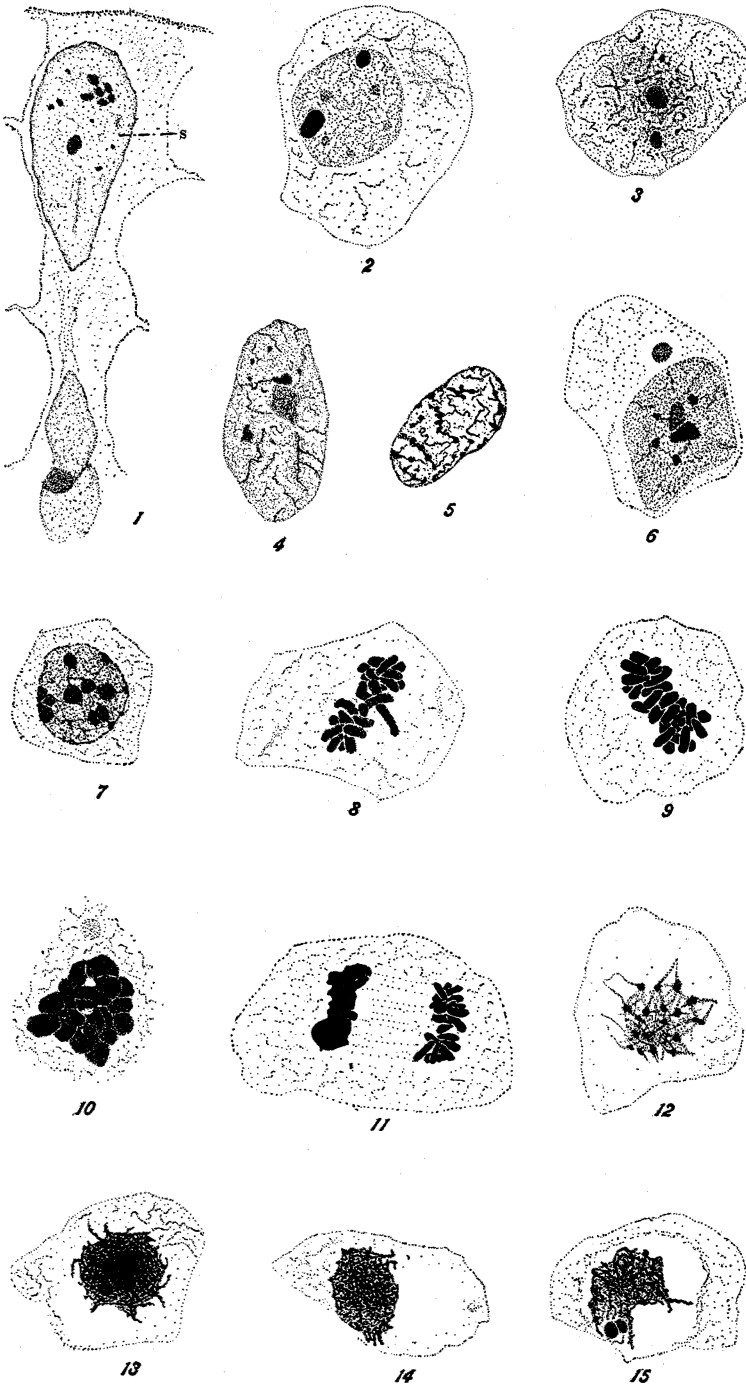
Fig. 7. In this stage a condensation of the nuclear material is taking place, forming masses which later transform directly into the chromosomes of the spermatogonia.

Figs. 8–10. Metaphase stages of the spermatogonia showing the probable twenty-two chromosomes. Fig. 10 is taken from a smear preparation.

Fig. 11. Shows the divided chromosomes passing towards the poles. The X and the Y elements cannot be identified at this stage.

Fig. 12. Prophase of the primary spermatocyte showing the chromosomes from the previous division weaving out into fine strands which immediately spread through the nucleus.

Figs. 13–15. Synezesis stages of the primary spermatocytes showing the massing of the fibers, accompanied by a slow condensation, as shown in Fig. 15. This also shows the two accessory elements, which retain their spherical shape, while the ordinary chromosomes weave out into the leptotene threads.



L. J. BACHHUBER.

PLATE II.

FIG. 16. Shows a further condensation of the leptotene threads, which finally results in the large spireme. This stage also shows the first appearance of the chromatoid body.

FIG. 17. The two accessory elements lie a little to one side of the condensing leptotene threads. The accessories can nearly always be identified in these stages.

FIGS. 18, 19. These stages show the leptotene threads coming out of the synezesis stage and giving evidence of a chiasmatype synapsis.

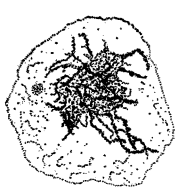
FIGS. 20, 21. The accessory elements are very conspicuous in the large spireme stages.

FIG. 22. Cell from a smear, showing evidence of a segmentation of the large spireme.

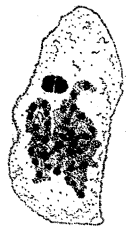
FIG. 23. The accessory elements lie a little to one side of the chromosomes massed in the equatorial plate stage, just previous to division. The chromatoid body has migrated into the same plane as the chromosomes.

FIG. 24. Shows the massing of the chromosomes, which usually hides the accessory elements. The chromatoid body is very prominent.

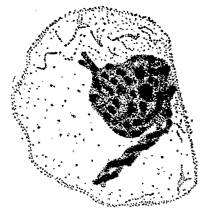
FIGS. 25–28. The two accessory chromosomes travel towards the poles long before the division of the ordinary chromosomes. The chromatoid body is still in the plane of the equatorial plate.



16

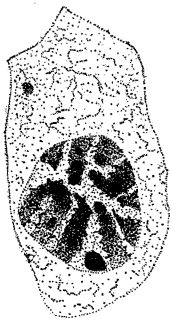


17



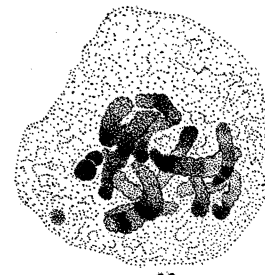
18

19

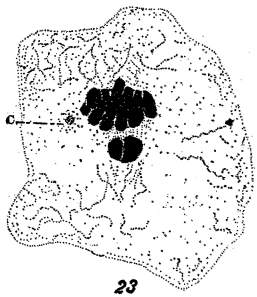


20

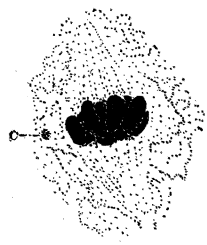
21



22

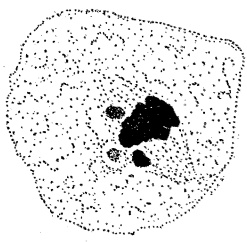


23

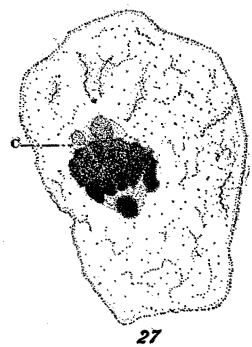


24

25



26



27

28

PLATE III.

FIG. 29. Again showing the accessory elements migrating towards the poles in advance of the ordinary chromosomes.

FIG. 30. A stage following division of the chromosomes of the primary spermatocyte showing the eleven chromosomes at the pole, the accessory in the center.

FIG. 31. Shows the chromatoid body migrating in between the two sets of chromosomes immediately after division.

FIGS. 32, 33. These cells give evidence of a precocious division of the X and the Y elements. In this case, two X elements and two Y elements travel towards the poles.

FIG. 34. The chromosomes of the secondary spermatocytes have immediately lined up in the equatorial plate stage, ready for the next division.

FIG. 35. The chromosomes of the secondary spermatocytes divide and pass to the poles in ring-like masses, practically losing their identity as individual elements. This stage also shows the chromatoid body.

FIG. 36. Shows the chromosomes after reaching the poles and before formation of the spermatids. The chromatoid body is still present.

FIGS. 37, 38. Show the occasional identification of the accessory elements in the center of the closely interwoven mass of chromosomes, giving a ring-like appearance.

FIGS. 39, 40. Condensation of nuclear material at the periphery of the nuclear wall. Also shows the presence of the chromatoid body.

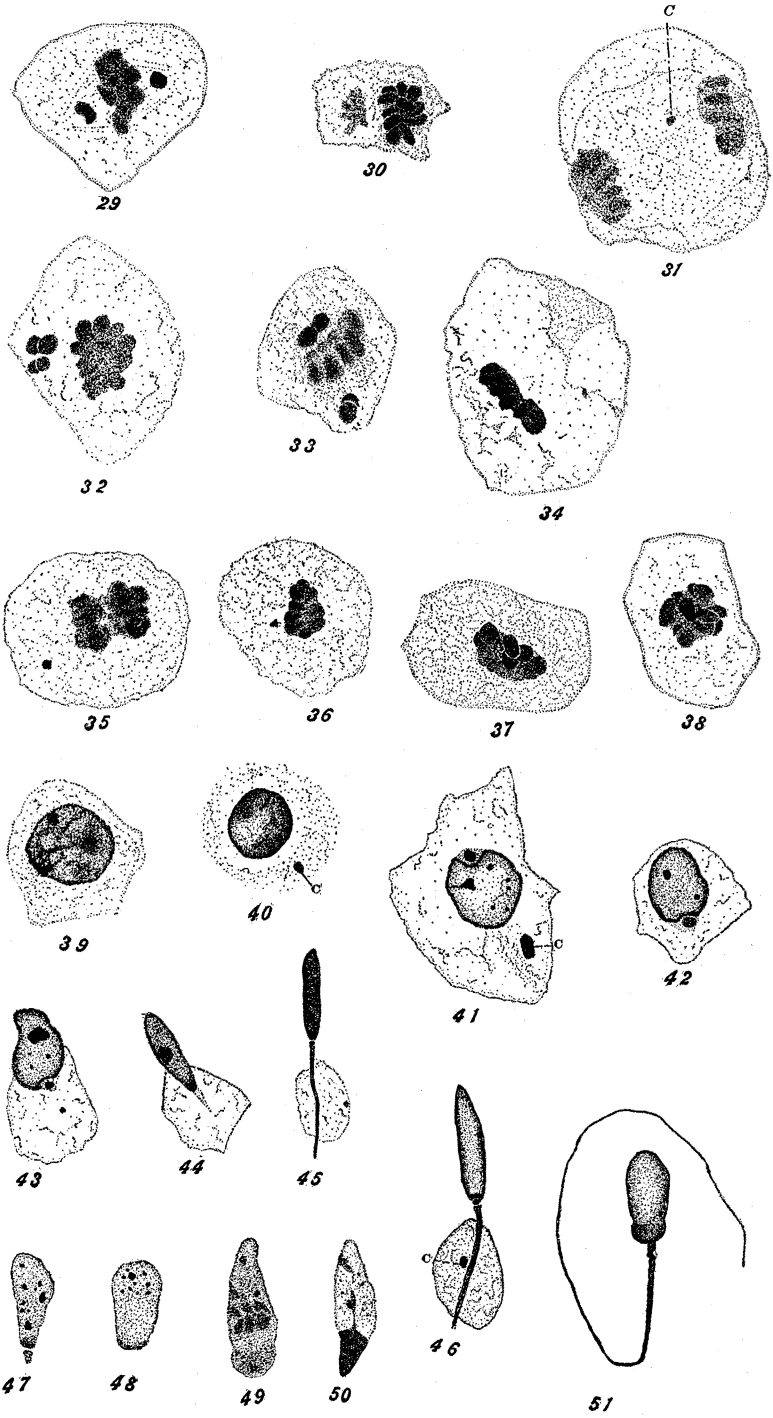
FIGS. 41–43. Further condensation of chromatin around the periphery of the nuclear wall. The centrosome is meshed in one side of the nucleus. The chromatoid body may lie in any portion of the cytoplasm and may be very irregular in shape.

FIGS. 44–46. Still further condensation, with the gradual escape of the nucleus from the excess cytoplasm. The chromatoid body is cast off with the excess cytoplasm.

FIGS. 47, 48. Show the presence of darker staining masses of chromatin material which may represent the individual chromosomes in the sperm head.

FIGS. 49, 50. The darker staining masses now diffuse through the sperm head and finally form an even-staining mass of chromatin.

FIG. 51. Fully developed spermatozoön, showing the head, the middle-piece with the centrosome, and the long, thin, lightly staining sperm tail.


C
29
30
31
32
33
34
35
36
37
38
39
40
C
41
C
42
43
44
45
46
C
47
48
49
50
51